

09/703,350

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	MEHRABAN ET AL.	Examiner:	G. NICKOL
Serial No.:	09/703,350	Group Art Unit:	1642
Filed:	OCTOBER 31, 2000	Docket No.:	11669.213USU1
Confirmation No.:	3065	Customer No.:	23552
Title:	DIFFERENTIALLY EXPRESSED GENES INVOLVED IN ANGIOGENESIS, THE POLYPEPTIDES ENCODED THEREBY, AND METHODS OF USING THE SAME		

CERTIFICATE UNDER 37 CFR 1.6(d):

I hereby certify that this paper is being transmitted by facsimile to the U.S. Patent and Trademark Office on January __, 2006.

By: _____
Name: Sheryl A. Boerboom

DECLARATION UNDER 37 C.F.R. § 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

We, Mary Gerritsen, Fuad Mehraban and Luca Rastelli declare and state:

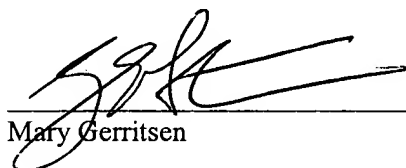
1. We are co-inventors of the subject matter of the above-identified application.
2. We understand that the Examiner has cited Olsen et al. (U.S. Publication No. 20020042372A1) which claims priority to a provisional application U.S. Serial No. 60/161,740 with a filing date of October 27, 1999.
3. We state that before October 27, 1999, we invented the subject matter described and claimed in the patent application Serial No. 09/703,350, filed October 31, 2000.
4. We understand that the claims of the patent application identified above are directed to methods of inhibiting angiogenesis in a mammal comprising administering to the mammal an effective amount of an antibody or antigen binding fragment that specifically binds to a polypeptide that comprises an amino acid sequence of SEQ ID NO:76 or an immunogenic fragment of a polypeptide comprising an amino acid sequence of SEQ ID NO:76.
5. Exhibit A describes that we understood that stanniocalcin was highly induced in human umbilical cord endothelial cells (HUVECs) and that its expression was blocked by a peroxisome proliferator-activated receptor γ (PPAR γ) ligand, 15dPGJ₂. The PPAR γ ligand, 15dPGJ₂, also inhibits tube formation. We further understood that stanniocalcin plays a role in either endothelial cell growth

and/or differentiation and/or in angiogenesis and angiogenic disorders. (Exhibit A1) Experiments carried out by Ms. Jeanne Kahn under the direction and control of Mary Gerritsen are shown in Exhibits A2, A3, A4, and A5. HUVECs were cultured either on collagen gels (Group I) or collagen films (Group II). The mRNA was isolated from HUVECs from each Group at 30 minutes, 2 hours, 4 hours, 8 hours, 16 hours, 24 hours, 38 hours, and 46.5 hours. (Exhibit A2) mRNA encoding stanniocalcin precursor was quantitated using TaqMan PCR. (Exhibit A3) mRNA encoding stanniocalcin precursor was increased as time of culture of HUVECs on collagen gels increased as compared to culture of HUVECs on collagen film. (Exhibit A3, A4 and A5) Culturing HUVECs on collagen gel, but not collagen film, leads to endothelial tube formation and is a model system for angiogenesis. The upregulation of mRNA encoding stanniocalcin precursor correlates with the endothelial tube cell formation of HUVECs cultured on collagen gels. The information provided in Exhibit A was obtained before October 27, 1999.

6. The evidence in Exhibit A shows that mRNA encoding a stanniocalcin precursor was isolated and was shown to be upregulated in HUVEC cells grown in collagen gels, which cells are known to be a model system for angiogenesis. The evidence in Exhibit A indicates that we invented the subject matter of the patent application identified above before October 27, 1999.

7. We further declare all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing therefrom.

Jan 10, 2006
date


Mary Gerritsen

date

Fuad Mehraban

date

Luca Rastelli



S/N 09/703,350

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6. The evidence in Exhibit A shows that mRNA encoding a stanniocalcin precursor was isolated and was shown to be upregulated in HUVEC cells grown in collagen gels, which cells are known to be a model system for angiogenesis. The evidence in Exhibit A indicates that we invented the subject matter of the patent application identified above before October 27, 1999.

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date

Mary Gerritsen

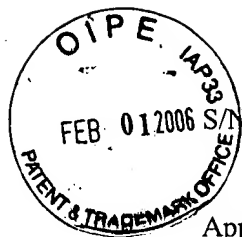
date

1-20-2006

Fuad Mehraban

date

Luca Rastelli



FEB 01 2006 S/N 09/703,350

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: MEHRABAN ET AL. Examiner: G. NICKOL
Serial No.: 09/703,350 Group Art Unit: 1642
Filed: OCTOBER 31, 2000 Docket No.: 11669.213USU1
Confirmation No.: 3065 Customer No.: 23552
Title: DIFFERENTIALLY EXPRESSED GENES INVOLVED IN
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METHODS OF USING THE SAME

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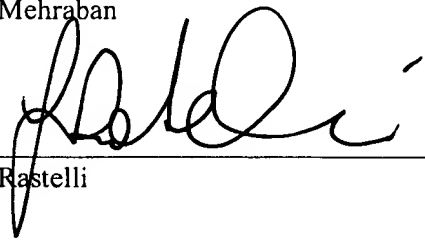
Mary Gerritsen


LR date

Fuad Mehraban

1-12-06

date



Luca Rastelli

NOTEBOOK NO. 28876

ISSUED TO Mary Gerritsen

ON _____ **19**

DEPARTMENT Cardiovascular Research

RETURNED _____ **19**

—SCIENTIFIC NOTEBOOK CO.—

2831 LAWRENCE AVE.

P.O. BOX 238

STEVENSVILLE, MI 49127

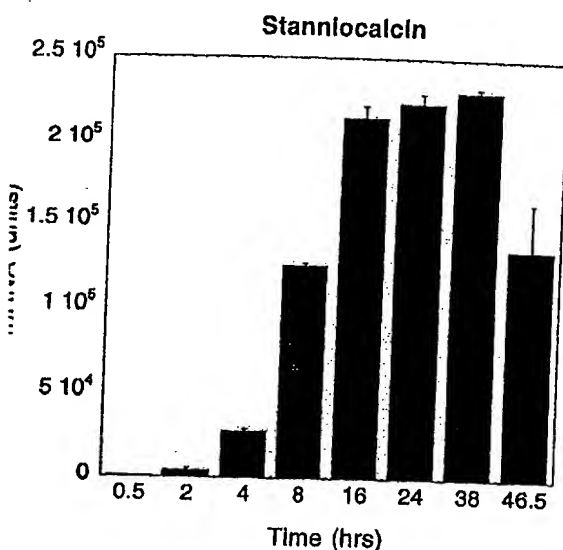
616-429-8285

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Statement of Idea

Several known genes have been identified from the curagen peritoneum as highly induced, including stanniocalcin, tissue factor protease inhibitor-2 (also known as placental protein 5), and plgf (placental growth factor). Of these stanniocalcin stands out as "unexpected" since its only known function relates to calcium phosphate transport in fish.

The observation that it is highly induced (see figure to left) and that its expression is blocked by PPAR γ ligand, 15d PGJ $_2$ (inhibits tube formation) suggests a possible role in the regulation of either endothelial cell growth and/or differentiation, and thus in angiogenesis & angiogenesis related disorders.



Plans - generate anti-peptide antibodies
 - clone & express protein
 & make monoclonal Abs
 - prepare probes for *in situ* hybridization

To Page No. _____

Processed & Understood by me,

Invented by

Recorded by

Date

From Page No. —

		1	2	3	4	5	6	7	8	9	10	11	12
Std. Curve - Stanniocalcin Precursor	A	✓ 250 ng 100 ng	✓ 50 ng 20 ng	✓ 10 ng 4 ng	✓ 2 ng 0.8 ng	✓ 0.4 ng 0.16 ng	✓ dH ₂ O NTC	✓ 100 ng of 200 ng RNA NRT					
Std. Curve - TFPI-2	B	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Stanniocalcin Precursor	C	Group 1 - 30 min.	✓ 2 hr.	✓ 4 hr.	✓ 8 hr.	✓ 16 hr.	✓ 24 hr.	✓	✓	✓	✓	✓	✓
Unknowns	D	38 hr.	✓ 46.5 hr.	✓ 30 min.	✓ 2 hr.	✓ 4 hr.	✓ 8 hr.	✓	✓	✓	✓	✓	✓
	E	✓ 16 hr.	✓ 24 hr.	✓ 38 hr.	✓ 46.5 hr.	✓ 100 ng	✓ 50 ng	✓ 10 ng	✓ 2 ng	✓	✓	✓	✓
TFPI-2 Unknowns	F	Group 1 - 30 min.	✓ 2 hr.	✓ 4 hr.	✓ 8 hr.	✓ 16 hr.	✓ 24 hr.	✓	✓	✓	✓	✓	✓
	G	✓ 38 hr.	✓ 46.5 hr.	✓ 30 min.	✓ 2 hr.	✓ 4 hr.	✓ 8 hr.	✓	✓	✓	✓	✓	✓
	H	✓ 16 hr.	✓ 24 hr.	✓ 38 hr.	✓ 46.5 hr.	✓ 100 ng	✓ 50 ng	✓ 10 ng	✓ 2 ng	✓	✓	✓	✓

Master Mix - 1X

RNA (Unknown RNAs @ 10 ng/μl)	5
10X Taqman Buffer A	- 10 μl
2.5 mM dNTP Mix	- 5 μl
25 mM MgCl ₂	- 6 μl
dH ₂ O	- 11 μl
50% Glycerol	- 10 μl
10 μM probe	- 0.5 μl
10 μM primers-for	- 0.5 μl
-rev	- 0.5 μl
RNasin (20 U/μl)	- 0.8 μl
AmpliTaq Gold (5 U/μl)	- 0.25 μl
*MuLV Rtase (50 U/μl)	- 0.25 μl
Total Volume = 50 μl	

*add after "NRT" controls are set up

100 master mix
X Rx Mix

Notes:

- ① RNA for Std. Curves = HUVEC #7, 1:1 dil. made on 50 ng/μl except 0.08 ng/μl sample made today @ 1:5 dil. of 0.08 ng/μl sample (200 μl RNA) 800 μl dH₂O 428 μl H₂O + 200 μl stock RNA (167 ng/μl)
- ② Used P10 pipettor to add RNAs today - New tubes of unk. RNAs (200 μl/tube) thawed today - (first thaw) left @ 4°C after today's run - will use all this wk. & hold @ 4°C during this time

- 4385 = 100 = 43.85 μl/Rx
① Take 200 μl of ea. gene (= 46 Rx.) being tested here & add enzymes/pro/primers as @ above - & then MuLV Rtase / ea.
② For unknown gene pro/primer being tested here, take 216.25 μl (= 5 Rx) of Master Mix & add 2.5 μl of ea. enzyme / Probe & primers - add 0.25 μl RTase

Witnessed & Understood by me,

JMS

Invented by

recorded by Jeanne W. Kaha

To Page No. 8

Page No. 88

Applied Biosystems Sequence Detection Systems 1.6.3

File: Standin_Precursor Plate Type: 7700 Single Reporter
PCR Volume: 50

Comments:

Real Cycle Conditions

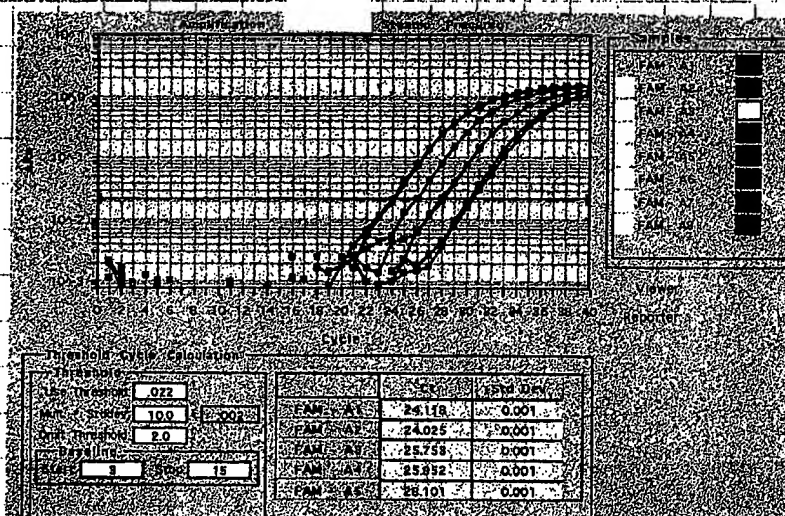
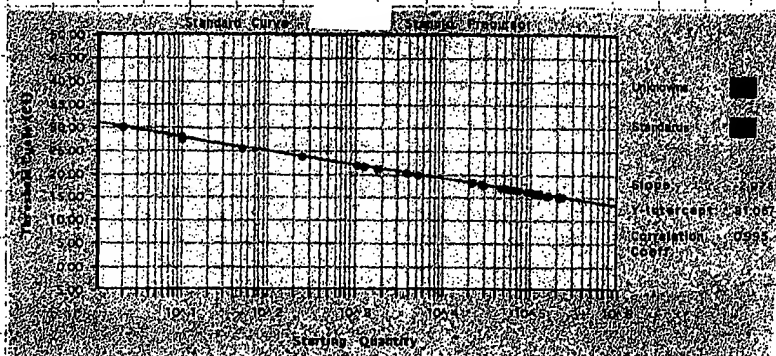
Temp	Time	Repeat	Ramp Time	Auto Increment
48.00	30:00		Auto	
95.00	10:00		Auto	
95.00	0:15	40	Auto	
60.00	1:00		Auto	
25.00	2:00		Auto	

Standard Curve

Slope: -2.98 Threshold: 0.02
Intercept: 31.07 Baseline Range: (8, 15)
Fit R: 1.00

Raw Information

Type	Sample Name	Replicate	Ct	Quantity	Std. Dev.	Mean
STND	Standinlocain		24.12	2.5e+02	0.00	250.00
STND	Standinlocain		24.02	2.5e+02	0.00	250.00
STND	Standinlocain		25.75	5.0e+01	0.00	50.00
STND	Standinlocain		25.95	5.0e+01	0.00	50.00
STND	Standinlocain		28.10	1.0e+01	0.00	10.00
STND	Standinlocain		27.91	1.0e+01	0.00	10.00
STND	Standinlocain		30.18	2.0e+00	0.00	2.00
STND	Standinlocain		30.89	2.0e+00	0.00	2.00
NTC	Standinlocain		40.00	0.00	0.00	0.00
NTT	Standinlocain		40.00	0.00	0.00	0.00
UNKN	Standinlocain		22.07	1.1e+03	0.00	1059.81
UNKN	Standinlocain		21.86	1.2e+03	0.00	1245.34
UNKN	Standinlocain		20.44	3.7e+03	0.00	8719.36
UNKN	Standinlocain		20.00	5.2e+03	0.00	5237.49
UNKN	Standinlocain		17.80	2.9e+04	0.00	28820.94
UNKN	Standinlocain		17.87	2.7e+04	0.00	27287.43
UNKN	Standinlocain		15.88	1.8e+05	0.00	126905.3
UNKN	Standinlocain		15.90	1.2e+05	0.00	124691.7
UNKN	Standinlocain		15.17	2.2e+05	0.00	219891.9
UNKN	Standinlocain		15.23	2.1e+05	0.00	210000.9
UNKN	Standinlocain		15.13	2.3e+05	0.00	227739.8
UNKN	Standinlocain		15.17	2.2e+05	0.00	219870.7
UNKN	Standinlocain		15.12	2.9e+05	0.00	229266.5
UNKN	Standinlocain		15.10	2.9e+05	0.00	232274.0
UNKN	Standinlocain		16.00	1.2e+05	0.00	115798.5
UNKN	Standinlocain		15.62	1.6e+05	0.00	155442.5
UNKN	Standinlocain		21.38	1.8e+03	0.00	1808.16
UNKN	Standinlocain		21.40	1.8e+03	0.00	1780.69
UNKN	Standinlocain		18.20	2.1e+04	0.00	21046.10
UNKN	Standinlocain		18.21	2.1e+04	0.00	20941.43
UNKN	Standinlocain		17.15	4.8e+04	0.00	47689.94
UNKN	Standinlocain		17.05	5.1e+04	0.00	51282.37
UNKN	Standinlocain		16.10	1.1e+05	0.00	106828.8
UNKN	Standinlocain		16.20	1.0e+05	0.00	99581.46
UNKN	Standinlocain		16.32	9.1e+04	0.00	90581.57
UNKN	Standinlocain		16.25	9.9e+04	0.00	95337.09
UNKN	Standinlocain		16.57	7.4e+04	0.00	74407.20
UNKN	Standinlocain		16.80	6.8e+04	0.00	62605.40
UNKN	Standinlocain		16.98	5.7e+04	0.00	56569.47
UNKN	Standinlocain		17.17	4.7e+04	0.00	48906.06
UNKN	Standinlocain		17.19	4.6e+04	0.00	46158.80
UNKN	Standinlocain		17.17	4.7e+04	0.00	46874.91



To Page No. 90

Used & Understood by me,

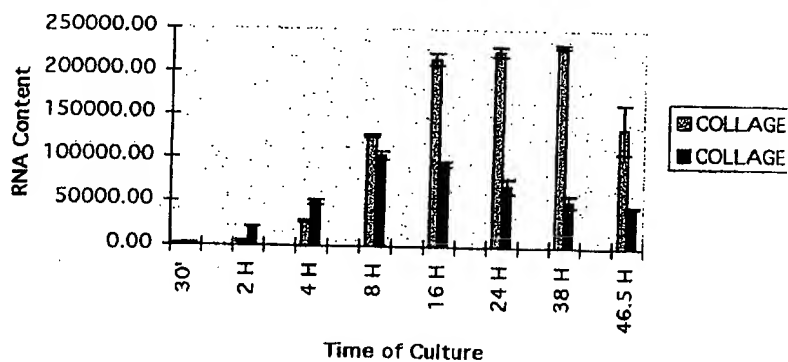
Invented by

Recorded by Seamus W. Kahn

Stanniocalcin Precurs.

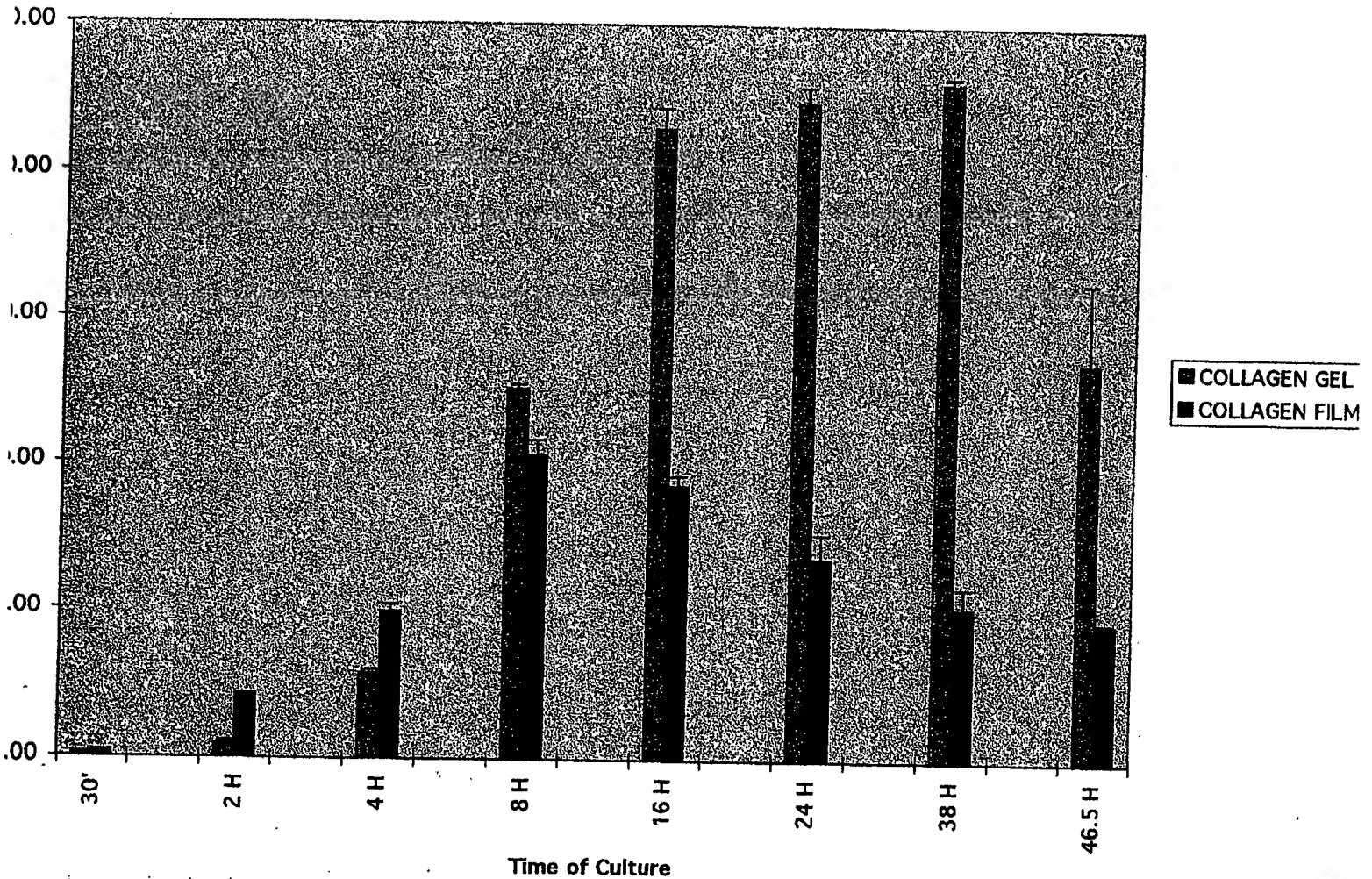
SAMPLE	Stannio. Pre.	AVERAGE	STDEV	TIME OF CULTURE	COLLAGEN GEL	COLLAGEN FILM	GROUP 1 STDEV	GROUP 2
GROUP 1-30'	1053.80	1149.55	135.41	30'	1149.55	1794.45	135.41	
	1245.30							
GROUP 1-2 H	3719.40	4478.45	1073.46	2 H	4478.45	20993.50	1073.46	
	5237.50							
GROUP 1-4 H	28820.00	28053.50	1083.99	4 H	28053.50	49486.00	1083.99	
	27287.00							
GROUP 1-8 H	126810.00	125750.00	1499.07	8 H	125750.00	103205.50	1499.07	
	124690.00							
GROUP 1-16 H	219890.00	214945.00	6993.29	16 H	214945.00	92959.50	6993.29	
	210000.00							
GROUP 1-24 H	227740.00	223805.00	5564.93	24 H	223805.00	68506.00	5564.93	
	219870.00							
GROUP 1-38 H	229270.00	230770.00	2121.32	38 H	230770.00	51738.00	2121.32	
	232270.00							
GROUP 1-46.5 H	115800.00	135620.00	28029.71	46.5 H	135620.00	46514.50	28029.71	
	155440.00							
GROUP 2-30'	1808.20	1794.45	19.45					
	1780.70							
GROUP 2-2 H	21046.00	20993.50	74.25					
	20941.00							
GROUP 2-4 H	47690.00	49486.00	2539.93					
	51282.00							
GROUP 2-8 H	106830.00	103205.50	5125.82					
	99581.00							
GROUP 2-16 H	90582.00	92959.50	3362.29					
	95337.00							
GROUP 2-24 H	74407.00	68506.00	8345.27					
	62605.00							
GROUP 2-38 H	56568.00	51738.00	6830.65					
	46908.00							
GROUP 2-46.5 H	46154.00	46514.50	509.82					
	46875.00							

Stanniocalcin Precursor



[Signature]

Stanniocalcin Precursor



[Signature]

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